## REMARKS

### Status of the Claims

Claims 1-3, 5-8, 10, 12, 14-29, 31-35, and 37-39 are currently pending in the present application. Claims 8 and 14-27 are withdrawn as directed to a non-elected invention. Claims 4, 9, 11, 13, 30, and 36 were previously canceled. Reconsideration in view of the following remarks is respectfully requested.

### Issues Under 35 U.S.C. § 103(a)

*Basis for the Rejection*

Claims 1-7, 10, 12, 28-29, 33-35, and 37-39

Claims 1-7, 10, 12, 28-29, 33-35, and 37-39 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Stohl *et al., The Journal of Immunology,* 1990, 144:3718-3725, ("Stohl") in view of Cardarelli *et al., Cellular Immunology,* 1991, 135:105-117, ("Cardarelli"), U.S. Patent No. 5,198,423 to Taguchi *et al.,* ("Taguchi"), Ybarrondo *et al., Immunology,* 1997, 91:186-172 and Neri *et al., Clinical and Diagnostic Laboratory Immunology,* 2001, 8:11311135, ("Neri"), *see* Office Action, pages 2-5. Applicants respectfully traverse.

Specifically, the Examiner states that Stohl describes a method of differentiating PBMCs with anti-CD3 and IL-2 to obtain cytotoxic T lymphocytes ("CTLs"), *see* Office Action, page 3. The Examiner admits that Stohl does not describe incubating the cells with recombinant fibronectin. However, according to the Examiner, Cardarelli and Ybarrondo remedy these deficiencies. In particular, the Examiner states that Cardarelli teaches enhancing the expansion of PBMCs using immobilized fibronectin, anti-CD3 antibody, and IL-2. The Examiner further states that Ybarrondo teaches that immobilized fibronectin enhances the degranulation (cytolytic activity) of cytotoxic lymphocytes.

The Examiner cites Taguchi because this reference allegedly teaches that recombinant fibronectin is advantageous in comparison to natural fibronectin. The Examiner cites Neri for describing a method of evaluating cytotoxic lymphocyte activity by labeling target cells with calcein-AM and detecting fluorescence released by lysed target cells.

The Examiner believes that an ordinary artisan would have combined the above described

references to achieve the instant invention. According to the Examiner, an ordinary artisan would have been motivated to combine the immobilized fibronectin described by Cardarelli with the method for differentiating PBMCs into CTLs, as described by Stohl, since Cardarelli teaches that fibronectin enhances the expansion of T cells, which are cultivated under conditions identical to those of Stohl. The Examiner believes that an ordinary artisan would have combined Ybarrondo and Stohl since Ybarrondo allegedly teaches that fibronectin enhances the degranulation response in CTLs.

The Examiner further notes that an ordinary artisan would have replaced the native fibronectin described in Cardarelli with the recombinant fibronectin fragment described in Taguchi since the Taguchi reference teaches fragments, which comprise the sequences taught by Cardarelli, as important to T cell stimulation. In addition, the Examiner states that the radioactive assay described in Stohl could have been replaced with the calcein-AM cytoxicity described by Neri, since Neri teaches that the calcein-AM assay is convenient and sensitive. For the reasons set forth below, Applicants respectfully disagree.

*Standard for Obviousness*

In the United States, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti,* 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

In addition, an "obvious to try" standard may not be applied, where one would have "to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful." *In re Kubin,* 561 F. 3d 1351 (Fed. Cir. 2009)

*A prima facie case of obviousness has not been established*

Stohl teaches that substantial toxicity is associated with LAK cells, *i.e.* white blood cells stimulated to kill tumor cells, and Interleukin-2 (IL-2) therapy, *see* page 3718, right column. The

purpose of Stohl's study was to generate potent cytolytic activity in the absence of exogenous IL-2, to assist in the development of more effective and less toxic clinical adoptive immunotherapy protocols, *see* abstract. To achieve this purpose, Stohl differentially induced cytolytic activity in PBMCs using three different anti-CD3 monoclonal antibodies, *see* page 3718, first full paragraph. Accordingly, the principle of operation of Stohl is to determine if PBMCs can be induced to exhibit different levels of cytolytic activity using different anti-CD3 antibodies. Modifying Stohl by adding fibronectin to the cultures to increase proliferation of PBMCs, as described in Cardarelli, would change the principle of operation of this reference from inducing cytolytic activity to increasing proliferation.

In addition, modifying the Stohl reference according to Ybarrondo would also change the principle of operation of Stohl. As noted above, Stohl's principle of operation is to determine if precursor CTLs, *i.e.* PBMCs, can be induced to exhibit different levels of cytolytic activity using different anti-CD3 antibodies. Ybarrondo teaches that cytolytic activity may be enhanced by adding fibronectin to cells, which already exhibit cytolytic activity. Modifying Stohl by adding fibronectin to CTL cultures is unrelated to Stohl's principle of operation, which concerns induction of precursor cells, not enhancement of CTLs.

Applicants further submit that the Examiner has not provided a reason as to why a skilled artisan would be led along a path that diverges from the principal and purpose of Stohl. Accordingly, the Examiner's rejection is little more than an improper "obvious to try" rejection.

The Examiner's asserted combinations, at best, lead to open-ended speculation on how the hypothetical embodiments proposed by the Examiner would operate, which is improper, *see In re Kubin,* 561 F. 3d 1351 (Fed. Cir. 2009). An ordinary artisan would be required to vary all of the parameters of Stohl to achieve the instant invention, and the cited references give no indication, *e.g.*, if cytolytic activity will be enhanced in PBMCs simultaneously contacted with fibronectin and anti-CD3 antibodies. Rather, Ybarrondo teach contacting differentiated PBMCs, *i.e.*, CTLs, with fibronectin to enhance cytolytic activity. Further, it is unclear from Cardarelli if any anti-CD3 antibody can be combined with fibronectin to enhance proliferation.

Neither Taguchi nor Neri remedy the deficiencies of Stohl, Ybarrondo, and Cardarelli. As noted above, Taguchi is merely cited for teaching that recombinant fibronectin is

advantageous in comparison to natural fibronectin. Neri is cited for describing a method of evaluating cytotoxic lymphocyte activity by labeling target cells with calcein-AM and detecting fluorescence released by lysed target cells. Accordingly, the claims are not rendered obvious by the cited references. Withdrawal of the rejection is respectfully requested.

Claims 31-32

Claims 31-32 are also rejected as allegedly obvious over Stohl, Cardarelli, Taguchi, Ybarrondo, and Neri in further view of Chen *et al.*, 1994, *J. Immunol.*, 153:3630-3638, ("Chen") *see* Office Action, page 5. Applicants respectfully traverse.

Specifically, the Examiner admits that Stohl, Cardarelli, Taguchi, Ybarrondo, and Neri do not teach or suggest transducing a foreign gene into the T cell. However, according to the Examiner, Chen remedies this deficiency. As Applicants noted above, the instant claims are not rendered obvious by the combination of Stohl, Cardarelli, Taguchi, Ybarrondo, and Neri. Chen is merely cited for describing the transduction of foreign genes into T cells. Accordingly, Chen fails to remedy deficiencies of Stohl, Cardarelli, Taguchi, Ybarrondo, and Neri. In view of the foregoing, withdrawal of the rejection is respectfully requested.

**Obviousness-Type Double Patenting**

*U.S. Application No. 10/486,512*

Claims 1-7, 10, 12, 28-29, 31-35, and 37-39, are provisionally rejected on the ground of obviousness-type double patenting as allegedly unpatentable over claims 1, 8, 15-16, 30, 32, 34, and 36-38 of co-pending U.S. Application No. 10/486,512 in view of Mizobata *et al., British J. Cancer,* 74(10):1598-1604 ("Mizobata"), Chen and Neri, *see* Office Action, pages 6-7. Applicants respectfully traverse.

The claims in the instant application are directed to a method for expanding cytotoxic lymphocytes, which comprise, *inter alia*, culturing precursor cells, *e.g.*, PBMCs, in the presence of recombinant fibronectin and interleukin-2 to obtain expanded cytotoxic lymphocytes that maintain cytotoxic activity longer than cytoxtoxic lymphocytes expanded in the absence of a least one fibronectin fragment. In contrast, the claims in U.S. Application No. 10/486,512 are

directed to a method for inducing a cytotoxic T cell having an antigen-specific cytotoxic activity, comprising, *inter alia,* incubating a precursor cell with an antigen presenting cell in the presence of a fibronectin fragment. Accordingly, U.S. Application No. 10/486,512 comprises an essential element, *i.e.*, an antigen presenting cell, which is not specified in the instant claims. Further, U.S. Application No. 10/486,512 does not describe using IL-2 for induction and expansion of precursor cells. Mizobata fails to remedy this deficiency. Unlike the instant claims, which describe the induction and expansion of cytotoxic lymphocytes from precursor cells, Mizobata describes inducing PBMCs with autologous, mixed lymphocyte tumor cultures, *see* Materials and Methods of Mizobata. Mizobata describes obtaining cytotoxic lymphocytes from culturing PBMCs with the tumor cells, which are subsequently contacted with IL-2. Neither Neri nor Chen remedy these deficiencies. These references are merely cited for describing methods of assessing lysis of target cells and transduction of foreign genes into T cells. In view of the foregoing, the claims in U.S. Application No. 10/486,512 do not render the instant claims obvious. Withdrawal of the rejection is respectfully requested.

*U.S. Application No. 10/568,745*

Claims 1-7, 10, 12, 28-29, 31-35, and 37-39 are provisionally rejected on the ground of non-statutory double patenting as allegedly obvious over claims 1-15 and 20-21 of co-pending U.S. Application No. 10/568,745 in view of Mizobata and Neri, *see* Office Action, pages 7-8. Applicants respectfully traverse.

As noted above, the claims in the instant application are directed to a method for expanding cytotoxic lymphocytes, which comprise, *inter alia*, culturing precursor cells, *e.g.*, PBMCs, in the presence of recombinant fibronectin and interleukin-2 to obtain expanded cytotoxic lymphocytes that maintain cytotoxic activity longer than cytoxtoxic lymphocytes expanded in the absence of a least one fibronectin fragment. In contrast, the claims in U.S. Application No. 10/568,745 are directed to a method for preparing a cytotoxic lymphocyte characterized in that the method comprises the step of carrying out at least one step selected from induction, maintenance, and expansion of a cytotoxic lymphocyte using a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume

in the presence of fibronectin. Accordingly, U.S. Application No. 10/568,745 comprises an essential element, *i.e.*, a medium containing 0% to less than 5% by volume of serum and plasma, which is not specified in the instant claims. Mizobata fails to remedy this deficiency.

In addition, unlike the instant claims, which describe the induction and expansion of cytotoxic lymphocytes from precursor cells, Mizobata describes inducing PBMCs with autologous, mixed lymphocyte tumor cultures, *see* Materials and Methods of Mizobata. In Mizobata, the cytotoxic lymphocytes obtained from culturing PBMCs with the tumor cells are subsequently contacted with the IL-2.

Neri does not remedy the deficiencies of U.S. Application No. 10/568,745 or Mizobata. Neri is merely cited for describing methods of assessing lysis of target cells. In view of the foregoing, the claims in U.S. Application No. 10/568,745 do not render the instant claims obvious.

Applicants further submit that that the instant application has an earlier filing date than that of U.S. Application 10/568,745. Applicants submit that the Examiner should withdraw the rejection in the instant application and address the provisional nonstatutory obviousness-type double patenting rejection in the later filed U.S. Application. According to the MPEP at § 804, if a "provisional" nonstatutory obviousness-type double patenting (ODP) rejection is the only rejection remaining in the earlier filed of the two pending applications, while the later-filed application is rejectable on other grounds, the Examiner should withdraw that rejection and permit the earlier-filed application to issue as a patent without a terminal disclaimer. In view of the foregoing, withdrawal of the rejection is respectfully requested.

## CONCLUSION

In view of the above, Applicants believe that the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact L. T. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: JAN 4 2010

Respectfully submitted,

By _____

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